Journal of Forensic and Legal Medicine 23 (2014) 44–48

Contents lists available at ScienceDirect

Journal of Forensic and Legal Medicine

journal homepage: www.elsevier.com/locate/jflm

Original communication

Cardiac histopathological and immunohistochemical changes due to electric injury in rats

Nagwa M. Ghandour, MD Assistant Professor [a], Abeer E. Refaiy, MD Assistant Professor [b], Ghada Ali Omran, PhD Lecturer [a,*]

[a] Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Assiut University, Assiut City 71524, Egypt
[b] Department of Pathology, Faculty of Medicine, Assiut University, Egypt

ARTICLE INFO

Article history:
Received 5 July 2013
Received in revised form
18 December 2013
Accepted 19 January 2014
Available online 28 January 2014

Keywords:
Myocardium
Immunohistochemistry
Histopathology
Experimental
Rats
Electric injury

ABSTRACT

It has been a puzzling forensic task to determine the cause of death as a result of electric shock in the absence of recognizable skin marks or definite postmortem morphological findings. In forensic pathology, while classical macroscopic and microscopic morphology remain core procedures to investigate deaths, a variety of subsidiary measures has been developed and incorporated to detail that pathology. C-fos, one of a small group of genes called primary response genes and its protein product, fos, are crucial elements of complex signaling mechanisms believed to be responsible for cell response to stimulation. It has been found that c-fos plays a significant role in myocardial lesions, and has close relation to injury repair of the molecule. The aim of this study was to detect the histopathological findings in the myocardium after fatal and non-fatal electrical injury in rats and to investigate the potential role of c-fos expression using immunohistochemistry to distinguish antemortem from postmortem electrocution. Forty adult female rats were implemented and randomly divided into four groups (A, B, C and D). Group (A) rats were subjected to instantaneous antemortem electricity and their hearts were collected either immediately (A_1) or after an hour (A_2) before being subjected to cervical dislocation. Group (B) rats were electrically injured instantaneously postmortem, hearts were collected immediately (B_1) or an hour later (B_2) while Group (C) rats were electrified up to death, and their hearts were also gathered either immediately (C_1) or after an hour (C_2) from electrocution. Lastly, another group of rats served as a control group (Group D). Subgroup (D_1): rats were clamped but not electrified, before death and another group of rats were clamped but not electrified, after being killed by cervical dislocation. Sections from the hearts of all groups were fixed in formalin and routinely processed. The c-fos oncogene expression was evaluated in all groups by immunohistochemistry. Significant histopathological findings were detected in groups A and C. Few c-fos oncogene protein positive cardiomyocyte nuclei were seen in rats of groups (A_1) and (B_1). Additionally, increased expression in rats of groups C_1, C_2 and A_2 were observed. On the other hand, no c-fos protein expression was seen either in the control (groups D_1 and D_2) or in group B_2. Significant differences ($p < 0.001$) in c-fos expression were observed among rats of groups with antemortem electric injury (A_1, A_2) and those of postmortem injury (B_1 and B_2). Thus, in addition to classical histopathological methods, c-fos can be regarded as a target in identifying electrical injury, and can be used as an indicator to distinguish antemortem from postmortem electric shock.

1. Introduction

It is a challenging forensic task to determine the cause of death in an electrocuted victim without detectable current marks on the skin.[1] In order to find an effective way for diagnosis of these cases, forensic pathologists have been making lot of efforts to resolve this problem.[2] It is a well-known fact that electricity can cause death or any degree of damage to various organs and systems according to the type, voltage and intensity of the electrical current and the location of damage. The electrical shock may strike the victim's central nervous system, the cardiovascular system, the skeletal muscular tissue, the lungs, the skin and other internal organs.[3] Cardiac arrest can also be induced by a number of mechanisms with little or no tissue damage.[4] The principal cause of death was described by Michiue and associates in 2009[5] as cardiac failure due to ventricular fibrillation caused by a direct effect of the electric current.

* Corresponding author. Tel.: +20 88 2413295.
E-mail addresses: ghada_ali@hotmail.com, ghada_ali@yahoo.com (G.A. Omran).

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http://dx.doi.org/10.1016/j.jflm.2014.01.007

In forensic pathology, while classical morphology remains a core procedure to investigate deaths, a spectrum of ancillary procedures has been developed and incorporated to detail the pathology.[6] C-fos, one of a small group of genes called primary response genes and its protein product, fos, are integral components of complex signaling mechanisms believed to be responsible for cell response to stimulation. The effects of many types of stimulation including drug-induced seizures, activation of receptors, growth factors, neuroactive drugs, electrical stimulation, and physiological states have been studied.[6] The expression of c-fos is known to be increased in particular diseases and pathophysiological processes, indicating that it may play a role in the pathogenesis of some diseases. In rat models of myocardial stunning (MS), the expression of fos protein increased apparently, i.e. c-fos plays a significant role in myocardial lesion, and has close relation to injury repair of the molecule.[7]

The aim of this study was to evaluate the effect of fatal and non-fatal electric injury in rats, to characterize the pattern of the structural myocardial changes after electric injury, to study the immunohistochemical expression of c-fos in heart and to evaluate if it could be used as an indicator to distinguish antemortem from postmortem electricity.

2. Materials and methods

2.1. Animal groups and experimental design

The experimental procedures were carried out after ethical approval according to the National Institute of Health Guidelines for Animal Care.[8] A total of 40 healthy female Wistar Albino rats of 4–5 months old (with average weight 200 ± 50 g) were recruited. The animals were maintained under temperature 22 °C, a 12 h light/dark cycle, ad libitum availability of pellet food and water. The experimental groups were randomly divided into four groups and dealt with as follow: rats subjected to antemortem electricity (group A), rats subjected to postmortem electricity (group B), the third group was exposed to electricity up to death (group C), lastly the control group (group D). The rats were subjected to electric current according to the method described by Wang et al.[9] Two metal clamps were connected to a pole of 220 V alternating current. One clamp was connected to rats left hind limbs and other to right forelimbs. All the animals in whole groups were anaesthetized via ether inhalation before being electrified and/or cervically dislocated. The rats within the control group were only anaesthetized before killing but not electrified.

Group (A): Ten rats were subjected to instantaneous (for 5 s) antemortem electricity. This group was divided randomly into two subgroups. Group (A_1): five rats were subjected to cervical dislocation and the hearts were collected *immediately*. Group (A_2): five rats *were left alive for* 1 h from electrical injury and then subjected to cervical dislocation before hearts collection

Group (B): Ten rats were electrically injured instantaneously (for 5 s) postmortem, after death by cervical dislocation. This group was divided randomly into two subgroups. Group (B_1): hearts were collected *immediately* in 5 rats. Group (B_2): hearts were collected after 1 h from electrical injury in the other 5 rats.

Group (C): Ten rats were electrified up to death, also divided randomly into two subgroups; each subgroup consisted of 5 rats. Group (C_1): hearts were collected *immediately*. Group (C_2): hearts were collected *after* 1 h after electrocution.

Group D (the control group): Ten rats were divided randomly into two subgroups; each subgroup consisted of 5 rats. Group (D_1): rats were clamped (for 10 s), but not electrified, *before* death by cervical dislocation. Group (D_2): were clamped (for 10 s), but not electrified, *after* being killed by cervical dislocation.

2.2. Histopathological and immunohistochemical examination

Sections from collected hearts were fixed in formalin and routinely processed. Five micrometer (μm) sections were cut and stained with Hematoxylin-Eosin (H&E). The tissue sections were observed under light microscope (Olympus, Tokyo, Japan) for detection of histopathological changes. Immunohistochemistry (IHC) was performed according to manufacturer's protocol and as previously described by Zhang et al.[8] Tissue sections (4-μm thick) of formalin-fixed, paraffin-embedded specimens were deparaffinized, rehydrated, and transferred to phosphate buffered saline (PBS; pH 7.6). The slides were rinsed twice with PBS, and then endogenous peroxidase was blocked by the hydrogen peroxide for 5 min. Antigen retrieval was done by boiling the slides in citrate buffer (pH 6) for 12 min. Then the slides were washed three times with PBS before being incubated overnight with c-fos rabbit polyclonal primary antibody (Cat No E4460 Spring Bioscience Ca USA) at a dilution of 1:50. The slides were then rinsed three times with PBS and incubated for 10 min at room temperature with the biotinylated goat antipolyvalent antibody (Thermo Scientific, Fremont, USA). After that, they were rinsed with PBS for three times and incubated for 10 min with streptavidin peroxidase (Thermo Scientific, Fremont, USA) at room temperature. Washing with PBS, and diaminibenzidine were applied for 5 min. Thereafter, the slides were rinsed in distilled water (DW), counterstained with Mayer's Hematoxylin, dehydrated and then mounted. Positive control for c-fos antibody was sections from skin as c-fos is expressed in nuclei of epidermis of skin and also in epithelium of sweet glands. Negative control slides were performed by omitting the primary antibody. A distinct brown nuclear staining was scored positive.

2.3. Interpretation of the histopathological findings

The term myofibre break-up included the following histological patterns as was described by Finechi et al.,[3]: (1) bundles of distended myocardial cells alternating with hyper-contracted cells. (2) Myocardial nuclei in the hyper-contracted cells have a "square" aspect rather than the ovoid morphology seen in distended myocytes. (3) hyper-contracted myocytes alternated with hyper-distended cells that are often divided by a widened disc. The above mentioned histopathologic changes were scored blindly for all rats belonging to electrocuted and control groups.

2.4. Interpretation of the immunohistochemical expression of c-fos

A brown nucleus indicated positive expression of c-fos oncogene protein in cardiomyocytes. Brown–yellow particles in the cytoplasm indicated positive expression of c-fos oncogene mRNA. The number of positive nuclei of five high-power fields was calculated under light microscope after Zhang et al.,[7] Counting was undertaken in 50 fields and the average was calculated.

2.5. Statistical analysis

All data were expressed as mean value ± standard deviation (SD). To analyze significant differences between groups one-way ANOVA followed by Tukey's post hoc test for multiple comparisons were employed. A probability level (p) < 0.05 was considered significant.

3. Results

3.1. Histopathology

Fig. 1 illustrates few foci of intramyocardial hemorrhage in 3 rats of group (A_1). Fig. 2 shows few square nuclei in all rats and thrombi in the intramyocardial vessels in 3 rats of group (A_2). Figs. 3,4 represents histopathological changes detected in group C: in the form of heamorrhagic areas in the myocardium in 6 rats, many square nuclei and bands of distended myocardial cells alternating with hypercontracted ones (myofibers break-up), which were obvious in 8 out of 10 rats of groups C_1 and C_2. Fig. 5 shows oval nuclei which were observed in all rats of control group (D). No histopathological abnormalities could be seen in myocardium of rats of group (B).

3.2. Immunohistochemistry

Table 1 shows immunohistochemical results of c-fos expression in the studied groups presented by mean ± SD. Few c-fos oncogene protein positive cardiomyocyte nuclei were seen in rats of groups A_1 and B_1 with means of 0.5 ± 0.48, 0.3 ± 0.53 respectively. Positive expression of c-fos protein increased in rats of groups C_1, C_2 and A_2 (4.1 ± 0.88, 2.7 ± 0.48 and 1.6 ± 0.52) respectively. Neither c-fos oncogene protein expression was detected in control group D nor in group B2. This coincided with the histopathological changes observed, as rats of group C were the most affected followed by rats of group A. A high significant differences ($p < 0.001$) in c-fos protein expression were observed between rats of A_1, A_2, C_1 and C_2. Also significant differences ($p < 0.001$) were seen between rats of A2, B1 and B2. While, less significant differences ($p < 0.02$) in c-fos oncogene protein expression were detected between groups B_1 and B_2. Figs. 6–8 displays few c-fos expression in group B_1, marked brown nuclei c-fos expression in cardiomyocytes in group C and negative c-fos expression in group D respectively.

4. Discussion

Death from electricity is predominantly physiological process, thus, the postmortem morphological findings are usually not evident and generally non-specific. The flow of electric current has specific effects on excitable tissues but typical morphological signs may be sparse or even absent.[1] Electric marks are found more frequently with high than low voltage current, and the circumstances may not indicate that electric current has passed through the body. This possible paucity of findings can cause considerable problems in the diagnosis of electrocution.[3] Regarding the pathological changes in the cardiac muscle in the present study, few square nuclei and thrombi in the intra-myocardial vessels were

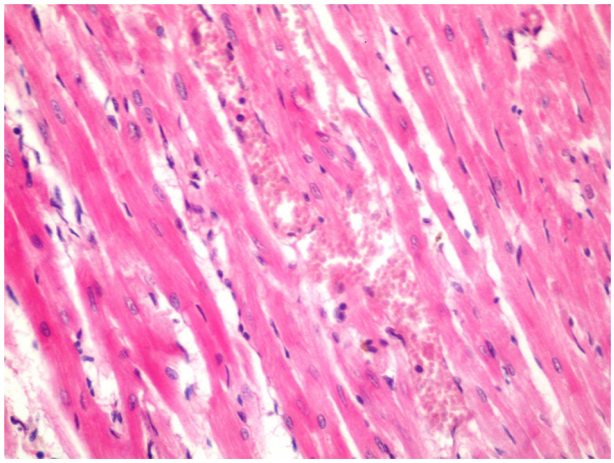

Fig. 1. A photo micrograph of a section from cardiac muscles of groups (A1, C1, C2) showing intramyocardial heamorrhage (H&E ×400).

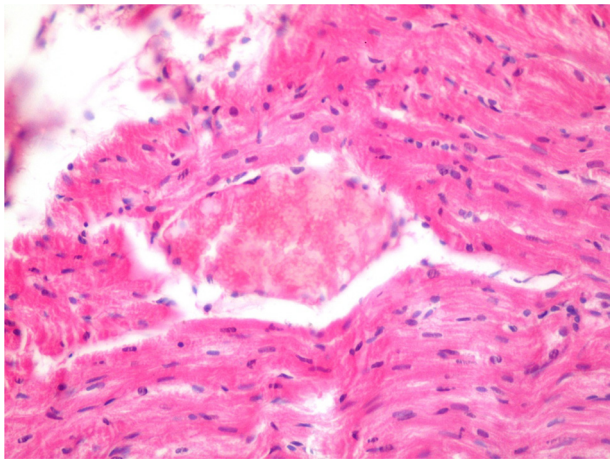

Fig. 2. A photo micrograph of a section from cardiac muscles of group (A2) showing thrombus in intramyocardial vessels (H&E ×400).

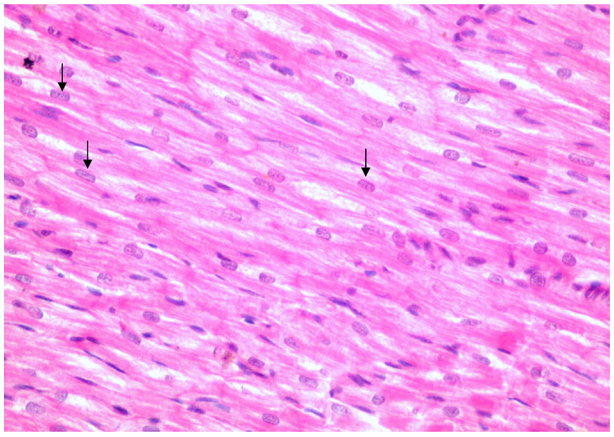

Fig. 3. A photo micrograph of a section from cardiac muscles of groups (C1, C2) showing hypercontracted myocells with many square nuclei (arrows) (H&E ×400).

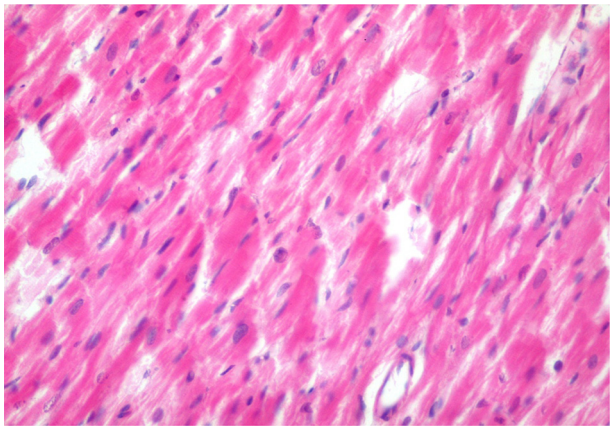

Fig. 4. A photo micrograph of a section from cardiac muscles of groups (C1, C2) showing hypercontracted myocytes alternating with hyper distended cells divided by widened disc. (H&E ×400).

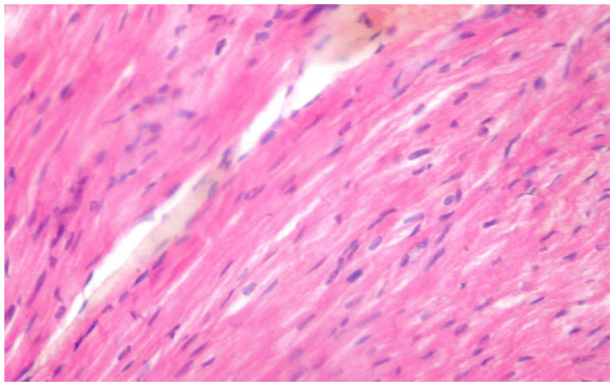

Fig. 5. A photo micrograph of a section from cardiac muscles of control group (D1, D2) showing oval nuclei (H&E ×400).

seen in rats of group (A_2). Hemorrhagic areas in the myocardium, many obvious square nuclei and bands of distended myocardial cells alternating with hypercontracted ones (myofiber break-up) were displayed in rats of group (C). While group (A_1), showed minimal changes; in the form of few foci of intramyocardial hemorrhage when compared to control (group D).

Jisheng[10] described formation of hypercontracted bands, rupture of intercalated disc, and shortening of myocomere, under electron microscope, in an animal model of cardiac damage after non-fatal electric injury and electrocution. Similarly, in an experimental model designed by Qin and his team,[11] rats were subjected to low voltage current. They observed ultrastructure changes of electricaly injured tissues in the form of hypercontracted bands in the myocardium. Break-up of myocardial fibers was also noticed in myocardium of 90% of electrocution cases examined by Fineschi and his co-worker.[12] The myofiber break-up described could be interpreted as a morphologic counterpart of a terminal dysfunction ending in ventricular fibrillation, giving a structural background to the electrical asynchronous activity and could be induced by the passage of abnormal electrical currents.[13]

In the present work, break-up of myocardial fibers was not found in any case electrified after death (B_1, B_2). This in agreement with Baroldi et al.,[13] who described its appearance to be vital antemortem change. On the other hand, Aggrawal[14] thought that the myofiber break-up may be perhaps a postmortem change. Vanderwee et al.[15] distinguished myofiber fragmentation due to knife motion (sometimes referred to as "chatter") in cutting histological sections from myofiber break-up. They also confirmed that similar changes were never described as part of rigor mortis of the myocardium. While others[16] described that only slight clumping of nuclear chromatin was observed in the myocardium after 1 h after death with dilation of the sarcoplasmic reticulum and contraction bands were seen 10 h later. Regarding the immunohistochemical (IHC) results in this study. Few c-fos oncogene protein positive cardiomyocyte nuclei were seen in rats of groups A_1 and B_1, this

Table 1
Immunohistochemical c-fos expression in different groups and subgroups of electrocuted and control rats.

Group	Mean ± SD
A1	0.5 ± 0.48
A2	1.6 ± 0.52
B1	0.3 ± 0.53
B2	0(−ve)
C1	4.1 ± 0.88
C2	2.7 ± 0.48
D1	0(−ve)
D2	0(−ve)

Mean values represents the number of c-fos oncogene protein positive cardiomyocyte's nuclei ± standard deviation. −ve means negative c-fos expression.

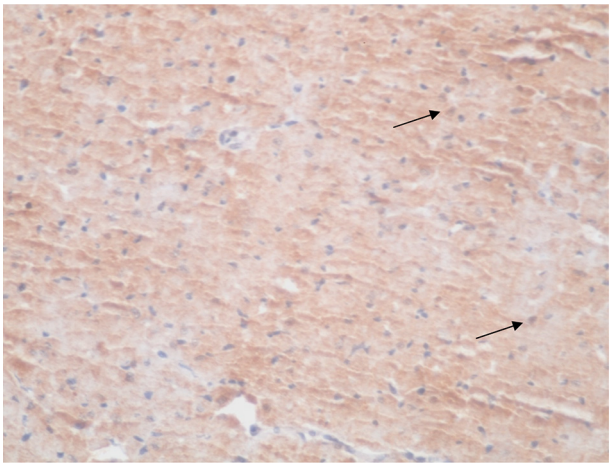

Fig. 6. A photo micrograph of a section from cardiac muscles of group (B1) showing c-fos expression as brown nuclei of cadiomycytes (arrows)(IHC ×200). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

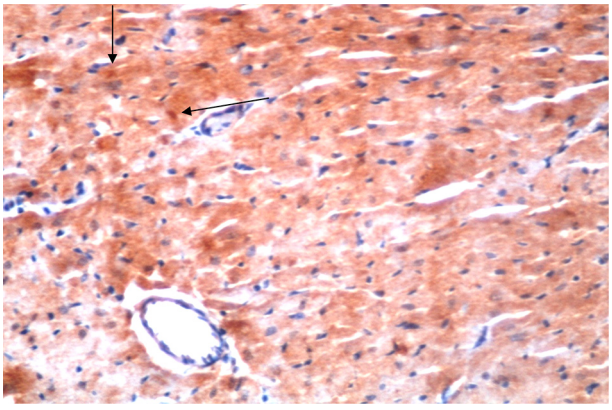

Fig. 7. A photo micrograph of a section from cardiac muscles of groups (C1,C2) showing brown nuclei (arrows) of c-fos expression in cardiomyocytes(IHC ×400). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

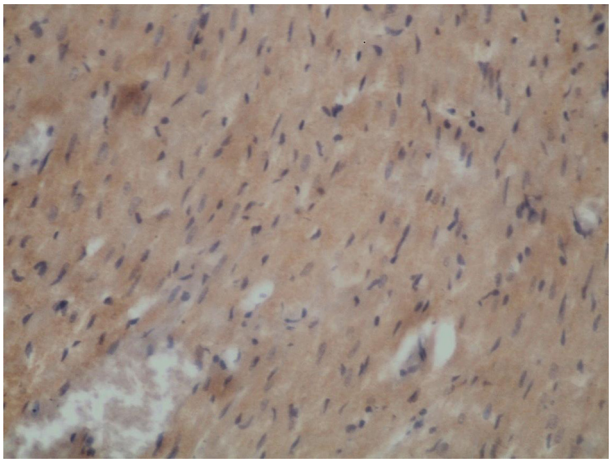

Fig. 8. A photo micrograph of a section from cardiac muscles of control group (D1,D2) showing negative expression of c-fos(IHC ×400).

could be explained as in some cases of cervical dislocation, the heart continued to beat sometimes for up to 20 min until hypoxia caused arrest.[17] Positive expression of c-fos protein increased in rats of groups C_1, C_2 and A_2 as c-fos plays an important role in cell response to stimulation and has close relation to injury repair of the molecule.[7,8] No c-fos oncogene protein expression was detected in the group B_2, while in B_1, they were few. Wang et al.[18] observed the expression of c-fos showed faintness in group of rats electrically injured immediately after death, and was negative in other rats that were electrified later after death.

Significant differences ($p < 0.001$) in c-fos oncogene protein expression were observed between rats of A_1, A_2, C_1 and C_2. Also significant differences ($p < 0.001$) were seen between rats of A_2, B_1 and B_2. This is in agreement with Wang et al. (2008 b), they found that the levels of c-fos mRNA in the antemortem electrocution group increased significantly compared with that of the postmortem electrocution group. From the aforementioned data, it was concluded that in absence of electrocution skin marks, the classical histology of the heart (H & E stained sections) can be a good substitute to investigate deaths due to electrical injury in forensic practice. Moreover, the immunohistochemical changes can provide an additional clue for the diagnosis. This study highlighted that c-fos expression can clearly discriminate between antemortem and postmortem injuries in the studied rat model. More studies should be carried out for measurement of c-fos in different pathological conditions, in different organs and in other species, which could be correlated with terminal electrocardiographic recordings.

Ethical approval

The Faculty of Medicine ethical committee, at Assiut University had granted the ethical permit for animal experimentation in this study.

Funding

None.

Conflicts of interest

None declared.

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